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Membrane Transport: A Coat for Ubiquitin

Dispatch

Michael J. Clague

Lysosomally directed receptors are concentrated at a 'bilayered' clathrin coat on the face of sorting endosomes. This coat is highly enriched in Hrs protein, which can potentially serve as an adaptor between ubiquitinated receptors and clathrin.

Upon stimulation, most cell surface receptors are internalised within clathrin coated vesicles, from which they are delivered to sorting endosomes. At this point, receptors may recycle to the cell surface or progress towards lysosomal compartments. Lysosomally directed receptors commonly get incorporated into small lumenal vesicles which pinch off from the limiting membrane of the sorting endosome, creating a so-called multivesicular body or pre-lysosomal compartment. Inclusion into these vesicles not only seals a receptor's fate, but also terminates signalling by sequestration from the cytosol.

Sorting endosomes exhibit complex morphologies which include tubular elements, proposed to be sites for recycling. These project from a vacuolar body, into which lumenal vesicles pinch off from the limiting membrane. It has been known for decades [1] that a significant fraction of the surface of the vacuolar sorting endosome is decorated with a flat clathrin coat, which until recently has received surprisingly little attention. Using electron microscopy, Martin Sachse and colleagues [2] have now produced a detailed examination of this coat structure, which has several unique characteristics that distinguish it from previously characterised clathrin coats. Typically, the coat presents as an extended flat surface which gives the impression of opposing the natural curvature of the membrane (Figure 1). It comprises two relatively electron dense layers, of which the inner layer is darkest, and has hence been termed a 'bilayered coat'. These dense layers are separated by a thin electron-lucent layer, which while characteristic is not always easy to discern.

Immunogold labelling revealed clathrin to be a coat component, but was unable to detect adaptor proteins that are associated with clathrin coats elsewhere in the cell. Quantification of receptor density in coated regions versus uncoated regions of the limiting membranes revealed that a recycling receptor — the transferrin receptor — was free to enter coated regions but not selectively enriched in this area. In contrast, the epidermal growth factor (EGF) receptor was enriched more than threefold in the 'bilayer-coated' region, indicating that it may have been retained by coat components. Could the coated region therefore represent the site of concentration of receptors prior to inclusion into lumenal vesicles?

Ubiquitination of receptors is now well established as a signal for sorting into lumenal vesicles of endosomes [3]. This suggests that an ideal adaptor protein for this coat might link ubiquitinated receptors to clathrin. Step forward Hrs, an endosomal protein that both binds clathrin and contains a ubiquitin interaction motif (UIM), which was first identified by bioinformatic analysis and has now been verified experimentally in a number of recent papers [4–9]. Moreover, Hrs is the orthologue of yeast Vps27, which belongs to the class E set of VPS genes that regulate multivesicular body formation [10].

The *Drosophila* homologue of Hrs has also been shown to be required for invagination of endosomal membranes [6]. Sachse *et al.* [2] found that Hrs is enriched 20-fold in the bilayer-coated regions of endosomes. Raiborg *et al.* [9] have taken things a step further; they have been able to direct transferrin receptor molecules away from recycling to the 'bilayer-coated' region by fusing ubiquitin to the receptor's carboxyl terminus. This fusion protein was also found to co-immunoprecipitate with Hrs — although it should be pointed out that this still does not formally show that the Hrs–receptor interaction is direct.

UIMs are also found in a number of proteins known to be involved with the endocytic pathway. These include the epsins Eps15 and Eps15R, which are believed to regulate receptor internalisation at the plasma membrane. The UIM was defined as a 20 amino acid stretch, on the basis of sequences of the S5a subunit of the proteasome which directly interact with ubiquitin [4]. One of the recent studies [5] has revealed that the UIM not only specifies ubiquitin binding, but is also necessary for mono-ubiquitination of the UIM-containing proteins themselves. Eps15, Eps15R, Hrs and epsins 1 and 2 were each shown to be mono-ubiquinated following activation of the EGF receptor [5]. This provides the scope both for amplification and the assembly of protein complexes by the recruitment of further ubiquitin-binding proteins. These may include the so-called ESCRT-1 complex, made up from the three class E proteins Vps23, Vps28 and Vps37, which is required for receptor sorting into lumenal vesicles of endosomes in yeast [11].

A component of the ESCRT-1 complex, Vps23, like its mammalian homologue TSG101, has a ubiquitin binding motif which is related to the active site of E2 ubiquitin-conjugating (Ubc) enzymes, but lacks the catalytic cysteine residue. Thus mono-ubiquitinated Hrs can, at least in principle, recruit TSG101 indirectly to ubiquitinated receptor molecules. The choreography of this endosomal budding reaction has become particularly compelling since the finding last year [12] that TSG101 is required in the budding and release from the plasma membrane of a number of enveloped viruses, including HIV-1 and Ebola. One topological

Physiological Laboratory, University of Liverpool, Crown Street, Liverpool, L69 3BX, UK.
E-mail: clague@liv.ac.uk

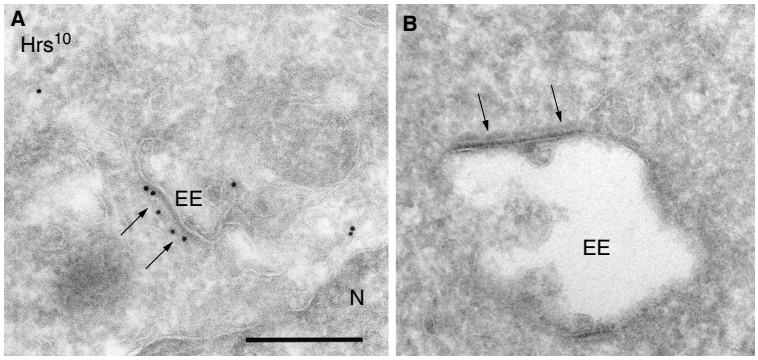

Figure 1. 'Bilayered' clathrin coats on endosomes.

(A) Hrs (10 nm gold) is concentrated in the 'bilayer' coated region of early endosomes (EE). Bar, 200 nm. (B) A clearer image of the endosomal clathrin coat which manifests as a flat lattice comprising 2 electron dense layers separated by a narrow electron lucent strip. (Reprinted with permission from [2].)

feature is conserved between viral budding and budding into endosomes: both involve budding away from the cytosol.

Lumenal vesicles in endosomes do not label with components of the external 'bilayered coat', at which cargo is concentrated. Sachse *et al.* [2] also report that they never see vesicle budding profiles beneath this 'bilayered coat', consistent with the visual impression that this area of the membrane is under tension. Instead, internal budding profiles are frequently seen at the edge of coated regions, leading to the suggestion that local coat disassembly may be required subsequent to receptor concentration for inward vesiculation to progress [2]. When tension is released from an elastic band it will bend in all directions — but one could imagine if tension is selectively released at an end, the rigid external coat would make involution more energetically favourable. How might coat disassembly be triggered? An intriguing suggestion comes from the finding that Hrs is tyrosine phosphorylated in response to stimulation of a number of growth factor and cytokine receptors. Following EGF stimulation this phosphorylation requires coincident localisation of both Hrs and receptor in endosomes. Upon EGF-induced phosphorylation, Hrs translocates to the cytosol, suggesting that this may play a role in regulated coat disassembly [13].

Hrs is likely to have other roles in co-ordinating receptor sorting. Its association with endosomes, at least in part by interaction of its FYVE domain with phosphatidyl inositol 3-phosphase (PI3P), provides one explanation for the requirement of PI 3-kinase hVPS34 in multivesicular body formation [14]. It also has a VHS domain, found in a number of proteins involved with membrane transport (Figure 2). In the best-studied case of GGA proteins, the VHS domain has been shown to bind cargo molecules directly. But if this is the case for the Hrs VHS domain, the recognition site must be somewhat different — the GGA–VHS/cargo interaction site has been determined structurally and is not conserved in Hrs [15,16]. Hrs interacts with a variety of other interesting proteins, including sorting nexin 1 (SNX1) and Merlin/NF2, in a regulated manner, but appears to be constitutively associated with Hbp/STAM2 [17]. Hbp in turn interacts through an SH3 domain with the de-ubiquitinating enzyme UBPY [18].

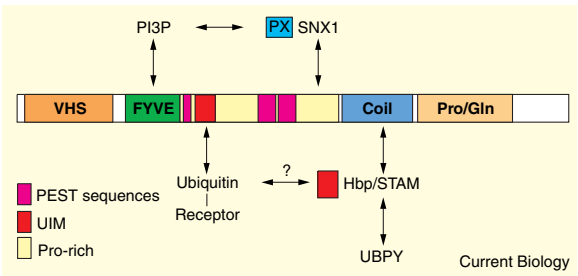

Figure 2. Hrs and its partners.

Domain structure of Hrs, showing interactions likely to be important for receptor sorting.

Although cargo deubiquitination is not mandatory for lumenal vesicle sorting, it is presumably required to maintain the levels of free cellular ubiquitin, without which the endocytic machinery will fail [19]. It may also play a role in disassembly of endosomal sorting complexes held together by networks of monoubiquitin interactions. Overproduction of Hrs alone leads to defects in receptor transport [13]; this may be due to competition with endogenous Hrs–Hbp complex and subsequent failure to recruit UBPY. In line with this interpretation, Hrs overproduction has been shown to lead to accumulation of ubiquitin at endosomes [7].

Hrs is emerging as a central player in endosomal sorting to lumenal vesicles. It is implicated in concentrating ubiquitinated receptors at 'bilayered clathrin coats', in the assembly and disassembly of these coats and, through indirect recruitment of UBPY, in the regeneration of free ubiquitin from receptors perhaps already selected for lysosomal destruction.

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